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(57) Abstract

Polynucleotides which encode the polypeptide MCP-4, as well as such polypeptides, antibodies and antagonist/inhibitors against the polypeptide and the use of the polypeptide and antagonist/inhibitors as pharmaceutical for treatment of tumors, wounds, parasitic infection, regulation of hematopoiesis, inflammation, rheumatoid arthritis, lung inflammation, allergies, atherosclerosis and infectious diseases such as tuberculosis.

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MONOCYTE CHEMOTACTIC PROTEIN-4

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptide of the present invention is monocyte chemotactic protein-4 (MCP-4). The invention also relates to inhibiting the action of such polypeptides.

There are three forms of monocyte chemotactic protein, namely, MCP-1, MCP-2 and MCP-3. All of these proteins have been structurally and functionally characterized and have also been cloned and expressed. MCP-1 and MCP-2 have the ability to attract leukocytes (monocytes, and leukocytes), while MCP-3 also attracts eosinophils and T lymphocytes (Dahinderi, E. et al., J. Exp. Med., 179:751-756 (1994)).

Initially, human monocyte-specific attracting factor, was purified from a glioma cell line and a monocytic cell line. Matsushima, K. et al, J. Exp. Med., 169:1485-1490 (1989). This factor was originally designated glioma-derived chemotactic factor (GDCF) and monocyte chemotactic and activating factor (MCAF) by Matsushima, et al. This factor is now referred to as MCP-1. Subsequent cloning of the cDNA for MCP-1 showed it to be highly similar to the murine JE gene. The JE gene could be massively induced in

murine fibroblasts by platelet-derived growth factor. Cochran, B.H., et al, Cell 33:939-947 (1983). Murine JE is highly similar to MCP-1. The MCP-1 protein is 62% identical to murine JE in a region of 68 shared N-terminal residues. It is widely accepted that JE and MCP-1 are species homologs. The polypeptide of the present invention, MCP-4, is both structurally and functionally related to MCP-1 and the murine JE protein, see Figure 2.

A method of suppressing tumor formation in a vertebrate by administering JE/MCP-1 has been disclosed in PCT application WO-92/20372, along with methods of treating localized complications of malignancies and methods of combatting parasitic infection by administering JE/MCP-1. Expression of the JE/MCP-1 protein in malignant cells was found to suppress the cells ability to form tumors *in vivo*.

Human MCP-1 is a basic peptide of 76 amino acids with a predicted molecular mass of 8,700 daltons. MCP-1 is inducibly expressed mainly in monocytes, endothelial cells and fibroblasts. Leonard, E.J. and Yoshimura, T., Immunol. Today, 11:97-101 (1990). The factors which induce this expression is IL-1, TNF or lipopolysaccharide treatment.

Other properties of MCP-1 include the ability to strongly activate mature human basophils in a pertussis toxin-sensitive manner. MCP-1 is a cytokine capable of directly inducing histamine release by basophils, (Bischoff, S.C. et al., J. Exp. Med., 175:1271-1275 (1992)). Furthermore, MCP-1 promotes the formation of leukotriene C4 by basophils pretreated with Interleukin 3, Interleukin 5, or granulocyte/macrophage colony-stimulating factor. MCP-1 induced basophil mediator release may play an important role in allergic inflammation and other pathologies expressing MCP-1.

Clones having a nucleotide sequence encoding a human monocyte chemotactic and activating factor (MCAF) reveal the primary structure of the MCAF polypeptide to be composed of a putative signal peptide sequence of 23 amino acid residues and a mature MCAF sequence of 76 amino acid residues. Furutani, Y.H., et al, Biochem. Biophys. Res.

Commu., 159:249-55 (1989). The compl te amino acid sequence of human glioma-derived monocyte chemotactic factor (GDCF-2) has also been determined. This peptide attracts human monocytes but not neutrophils. It was established that GDCF-2 comprises 76 amino acid r sidues. The peptide chain contains 4 half-cysteines, at positions 11, 12, 36 and 52, which create a pair of loops, clustered at the disulfide bridges. Further, the MCP-1 gene has been designated to human chromosome 17. Mehrabian, M.R., et al, Genomics, 9:200-3 (1991). Certain data suggests that a potential role for MCP-1 is mediating monocytic infiltration of the artery wall. Monocytes appear to be central to atherogenesis both as the progenitors of foam cells and as a potential source of growth factors mediating intimal hyperplasia. Nelken, N.A., et al, J. Clin. Invest., 88:1121-7 (1991). It has also been found that synovial production of MCP-1 may play an important role in the recruitment of mononuclear phagocytes during inflammation associated with rheumatoid arthritis and that synovial tissue macrophages are the dominant source of this cytokine. MCP-1 levels were found to be significantly higher in synovial fluid from rheumatoid arthritis patients compared to synovial fluid from osteoarthritis patients or from patients with other arthritides. Koch, A.E., et al, J. Clin. Invest., 90:772-9 (1992).

MCP-2 and MCP-3 are classified in a subfamily of proinflammatory proteins and are functionally related to MCP-1 because they specifically attract monocytes, but not neutrophils. Van Damme, J., et al, J. Exp. Med., 176:59-65 (1992). MCP-3 shows 71% and 58% amino acid homology to MCP-1 and MCP-2 respectively. MCP-3 is an inflammatory cytokine that regulates macrophage functions.

In accordance with one aspect of the present invention, there is provided a novel mature polypeptide which is MCP-4, as well as fragments, analogs and derivatives thereof. The polypeptide of the present invention is of human origin.

In accordance with another aspect of the present invention, there are provided polynucleotides (DNA or RNA) which encode such polypeptides.

In accordance with yet a further aspect of the present invention, there is provided a process for producing such polypeptide by recombinant techniques.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptide, or polynucleotide encoding such polypeptide for therapeutic purposes, for example, to treat tumors, to promote wound healing, to combat parasitic infection and to regulate hematopoiesis.

In accordance with yet a further aspect of the present invention, there is provided an antibody against such polypeptides.

In accordance with yet another aspect of the present invention, there are provided antagonist/inhibitors to such polypeptides, which may be used to inhibit the action of such polypeptides for therapeutic purposes, for example, to treat rheumatoid arthritis, lung inflammation, allergy, infectious diseases and to prevent inflammation and atherosclerosis.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

FIG. 1 depicts the cDNA sequence and corresponding deduced amino acid sequence of MCP-4. The 119 amino acid sequence shown is the full length protein, with approximately the first 22 amino acids representing a leader sequence such that the mature form of the protein is 97 amino acids in length. The standard one letter abbreviation for amino acids is used.

FIG. 2 illustrates the cDNA sequence homology between MCP-4 and the murine JE protein. The top sequence in each

three segments is MCP-4 and the bottom s quence is murine JE protein.

FIG. 3 shows the results of a Northern blot analysis of the mRNA transcript for MCP-4 in human cells.

Figure 4 shows the banding pattern of human MCP-4 following bacterial expression and purification.

Figure 5 is a schematic representation of the pQE-9 vector.

In accordance with an aspect of the present invention, there is provided an isolated nucleic acid (polynucleotide) which encodes for the mature polypeptide having the deduced amino acid sequence of Figure 1 or for the mature polypeptide encoded by the cDNA of the clone deposited as ATCC Deposit No. 75703 on March 10, 1994.

The polynucleotide of this invention was discovered from an activated monocyte cDNA library. It contains an open reading frame encoding a protein of approximately 119 amino acids in length of which the first 22 amino residues comprise a putative leader sequence. The mature protein therefore is predicted to be 97 amino acids in length. It is structurally related to mouse monocyte chemotactic protein (MCP-1 or JE), showing 27% identity, and 56% similarity over the entire human MCP-1 protein sequence. The polypeptide contains all four cysteine residues that occur in all chemokines in a characteristic motif. The spacing between these cysteines is conserved compared with the murine MCP-1/JE which strongly suggests that the new gene is a chemokine.

The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptide may be identical to the coding sequence shown in Figure 1 or that of the deposited clone or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the

same, mature polypeptide as the DNA of Figure 1 or the deposited cDNA.

The polynucleotide which encodes for the mature polypeptide of Figure 1 or for the mature polypeptide encoded by the deposited cDNA may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence such as a leader or secretory sequence or a proprotein sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of Figure 1 or the polypeptide encoded by the cDNA of the deposited clone. The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same mature polypeptide as shown in Figure 1 or the same mature polypeptide encoded by the cDNA of the deposited clone as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptide of Figure 1 or the polypeptide encoded by the cDNA of the deposited clone. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotide may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in Figure 1 or of the coding sequence of the deposited clone. As known in the

art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The present invention also includes polynucleotides, wherein the coding sequence for the mature polypeptide may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence is a preprotein and may have the leader sequence cleaved by the host cell to form the mature form of the polypeptide. The polynucleotides may also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains.

Thus, for example, the polynucleotide of the present invention may encode for a mature protein, or for a protein having a prosequence or for a protein having both a prosequence and a presequence (leader sequence).

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The present invention further relates to polynucleotides which hybridize to the hereinabove-

described sequences if there is at least 50% and preferably 70% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides . As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNA of Figure 1 or the deposited cDNA.

The deposit(s) referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

The present invention further relates to a MCP-4 polypeptide which has the deduced amino acid sequence of Figure 1 or which has the amino acid sequence encoded by the deposited cDNA, as well as fragments, analogs and derivatives of such polypeptide.

The terms "fragment," "derivative" and "analog" when referring to the polypeptide of Figure 1 or that encoded by the deposited cDNA, means a polypeptide which retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

The fragment, derivative or analog of the polypeptide of Figure 1 or that encoded by the deposited cDNA may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The present invention also relates to vectors which include polynucleotides of the present invention, host

cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the MCP-4 genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing polypeptides by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp,

the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila and Sf9; animal cells such as CHO, COS or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK,

pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are PKK232-8 and PCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation. (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook,

et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if

desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites,

polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The polypeptide can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. It is preferred to have low concentrations (approximately 0.15-5 mM) of calcium ion present during purification. (Price et al., J. Biol. Chem., 244:917 (1969)). Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

The polypeptides of the present invention, in particular MCP-4, may be used for the promotion of wound healing. Since MCP-4 is a chemokine, it is a chemo-attractant for leukocytes (such as monocytes, T lymphocytes, basophils, etc.); therefore, it causes infiltration of target immune cells to a wound area.

The MCP-4 polypeptides may also be used as an anti-tumor treatment and for treating localized complications of a malignancy, such as pleural effusions or ascites.

Instilling MCP-4 into the involved anatomic space can lead to local monocyte accumulation and activation.

The presence of MCPs *in vivo* is accompanied by a local increase in the presence of eosinophils which have the distinctive function of killing the larvae of parasites that invade tissues, as in schistosomiasis, trichinosis and ascariasis. Therefore, MCP-4 may be used for combatting parasitic infections.

MCP-4 polypeptides may also play a role in the regulation of hematopoiesis, by regulating various hematopoietic progenitor cell activation and differentiation.

The polypeptides may also be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as "gene therapy."

For example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding an MCP-4 polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered *in vivo* for expression of an MCP-4 polypeptide *in vivo* by, for example, procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells *in vivo* after combination with a suitable delivery vehicle.

The polypeptides of the present invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the polypeptide, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

The pharmaceutical compositions may be administered in a convenient manner such as by the topical, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. MCP-4 is administered in an amount which is effective for treating and/or prophylaxis of the specific indication. The amounts and dosage regimens of MCP-4 administered to a subject will depend on a number of factors such as the mode of administration, the nature of the condition being treated and the judgment of the prescribing physician. In general, the MCP-4 will be administered in an amount of at least about 10 μg/kg body weight and in most cases they will be administered in an amount not in excess of about 8 mg/Kg body weight per day. In most cases, the dosage is from about 10 μg/kg to about 1 mg/kg body weight daily, taking into account the routes of administration, symptoms, etc.

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a

particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the cDNA is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clones to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 500 or 600 bases; however, clones larger than 2,000 bp have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. FISH requires use of the clones from which the EST was derived, and the longer the better. For example, 2,000 bp is good,

4,000 is better, and more than 4,000 is probably not necessary to get good results a reasonable percentage of the time. For a review of this technique, see Verma et al., Human Chromosomes: a Manual of Basic Techniques, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that cDNA sequence. Ultimately, complete sequencing of genes from several individuals is required to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or

monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention.

The present invention also relates to a diagnostic assay for detecting the level of MCP-4 both quantitatively and qualitatively. Such assays are well known in the art and include an ELISA assay and the radioimmunoassay. The levels of MCP-4 detected in the assay can be useful for the elucidation of the significance of MCP-4 in various diseases and for the diagnosis of diseases in which MCP-4 may play a role.

This invention provides a method for identification of MCP-4 receptors. The gene encoding an MCP-4 receptor can be identified by expression cloning. Briefly, polyadenylated RNA is prepared from a cell responsive to MCP-4 and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to MCP-4. Transfected cells which are grown on glass slides are exposed to labeled MCP-4. MCP-4 can be labeled by a variety of means including iodidation or inclusion of a recognition site for a site-specific protein kinase. Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and retransfected using an iterative sub-pooling and rescreening process, eventually yielding a single clone that encodes the putative receptor. As an alternative approach for receptor identification, labeled MCP-4 can be photoaffinity linked with cell membrane or extract preparations that express an MCP-4 receptor molecule. Cross-linked material is resolved by PAGE and exposed to x-ray film. The labeled complex containing the MCP-4 receptor can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of generate oligonucleotide probes to screen a cDNA library to identify a gene encoding the putative receptor.

This invention also provides a method of screening drugs to identify those which enhance (agonists) or block (antagonists) interaction of MCP-4 to its receptor. An agonist increases the biological functions of MCP-4, while an antagonist reduces or eliminates such functions. As an example, a mammalian cell or membrane preparation expressing an MCP-4 receptor would be incubated with labeled MCP-4 in the presence of drug. The ability of drug to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of MCP-4 and its receptor would be measured compared in the presence or

absence of drug. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

The present invention is also directed to antagonist/inhibitor molecules to the polypeptides of the present invention. Antagonists include negative dominant mutants of MCP-4. MCP-4 is a tetrameric polypeptide wherein one mutated unit will cause the entire polypeptide to be non-functional. A negative dominant mutant of MCP-4 binds to the MCP-4 receptor but fails to activate cells (leukocytes and monocytes) to which it binds. An assay to detect negative dominant mutants of MCP-4 is an *in vitro* chemotaxis assay wherein a multiwell chemotaxis chamber equipped with polyvinylpyrrolidone-free polycarbonate membranes is used to measure the chemoattractant ability of MCP-4 for leukocytes in the presence and absence of potential antagonist/inhibitor or agonist molecules.

An example of an inhibitor is an antisense DNA or RNA construct. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the mature polypeptides of the present invention, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix -see Lee et al., Nucl. Acids Res., 6:3073 (1979); Cooney et al, Science, 241:456 (1988); and Dervan et al., Science, 251: 1360 (1991)), thereby preventing transcription and the production of MCP-4. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into the MCP-4 (antisense - Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Alternatively, antisense RNA and DNA

may be delivered to cells such that they are expressed *in vivo* to inhibit production of MCP-4.

Another example of an antagonist is a peptide derivative of MCP-4 which are naturally or synthetically modified analogs of MCP-4 that have lost biological function yet still recognize and bind to receptors thereby effectively blocking the receptors.

The antagonist/inhibitors may be used to treat inflammation by preventing the attraction of monocytes to a wound or a site of trauma, and to regulate normal pulmonary macrophage populations, since acute and chronic inflammatory pulmonary diseases are associated with sequestration of mononuclear phagocytes in the lung. They may also be used to treat rheumatoid arthritis, since MCP levels were found to be significantly elevated in synovial fluid from rheumatoid arthritis patients which suggests that synovial production of MCP attracts monocytes whose influx and activation are important in the pathogenesis of both degenerative and inflammatory arthropathies.

The antagonist/inhibitors may also be used for treating atherosclerosis, since MCPs mediate monocyte infiltration in the artery wall which infiltration leads to atherosclerosis, and to prevent allergies, since it has been shown that MCPs directly induce histamine release by basophils.

Antagonist/inhibitors may also be used to treat infectious diseases such as tuberculosis, since tuberculosis targets cells, usually monocytes, causing the monocytes to release MCPs which attracts more monocytes to the lungs causing severe inflammation. The antagonist/inhibitors may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinabove described.

The present invention also relates to an assay for identifying potential antagonist/inhibitors specific to MCP-4. An example of such an assay combines MCP-4 and a potential antagonist/inhibitor with membrane-bound MCP-4 receptors or recombinant MCP-4 receptors under appropriat

conditions for a competitive inhibition assay. MCP-4 can be labeled, such as by radioactivity, such that the number of MCP-4 molecules bound to the receptor can determine the effectiveness of the potential antagonist/inhibitor.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is directly on a polyacrylamide gel to isolate345Xelectr the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., Virology, 52:456-457 (1973).

Example 1

Bacterial Expression and Purification of MCP-4

The DNA sequence encoding for MCP-4, ATCC # 75703, is initially amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the processed MCP-4 protein (minus the signal peptide sequence) and the vector sequences 3' to the MCP-4 gene. Additional nucleotides corresponding to MCP-4 were added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5'-TCAGGATCCCCTACGGGCTCGTGGTC-3' contains a Bam H1 restriction enzyme site followed by 18 nucleotides of MCP-4 coding sequence starting from the presumed terminal amino acid of the processed protein codon. The 3' sequence 3'-CGCTCTAGAGTAAAACGACGGCCAGT-5' contains complementary sequences to the XbaI site and to a pBluescript SK- vector sequence located 3' to the MCP-4 DNA

insert. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). pQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-9 was then digested with Bam H1 and Xba I. The amplified sequences were ligated into pQE-9 and were inserted in frame with the sequence encoding for the histidine tag and the RBS. Figure 5 shows a schematic representation of this arrangement. The ligation mixture was then used to transform E. coli strain m15/rep4 available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, 1989. M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies were selected. Plasmid DNA was isolated and confirmed by restriction analysis. Clones containing the desired constructs were grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells were grown to an optical density 600 ($O.D._{600}$) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") was then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells were grown an extra 3 to 4 hours. Cells were then harvested by centrifugation. The cell pellet was solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized MCP-4 was purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag. Hochuli, E. et al., J.

Chromatography 411:177-184 (1984). MCP-4 (95% pure) was eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar glutathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein was dialyzed to 10 mmolar sodium phosphate. Figure 4.

Example 2

Expression pattern of MCP-4 in human cells

Northern blot analysis was carried out to examine the levels of expression of MCP-4 in human cells. Total cellular RNA samples were isolated with RNAzol™ B system (Biotecx Laboratories, Inc. 6023 South Loop East, Houston, TX 77033). About 10μg of total RNA isolated from each human tissue specified was separated on 1% agarose gel and blotted onto a nylon filter. (Sambrook, Fritsch, and Maniatis, Molecular Cloning, Cold Spring Harbor Press, (1989)). The labeling reaction was done according to the Stratagene Prime-It kit with 50ng DNA fragment. The labeled DNA was purified with a Select-G-50 column. (5 Prime - 3 Prime, Inc. 5603 Arapahoe Road, Boulder, CO 80303). The filter was then hybridized with radioactive labeled full length MCP-4 gene at 1,000,000 cpm/ml in 0.5 M $NaPO_4$, pH 7.4 and 7% SDS overnight at 65°C. After wash twice at room temperature and twice at 60°C with 0.5 x SSC, 0.1% SDS, the filter was then exposed at -70°C overnight with an intensifying screen. The message RNA for MCP-4 is abundant in activated and unactivated T cells, monocytes and T cell lines. Figure 3.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: LI, ET AL.

(ii) TITLE OF INVENTION: Monocyte Chemotactic Protein-4

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: CARELLA, BYRNE, BAIN, GILFILLAN, CECCHI, STEWART & OLSTEIN

(B) STREET: 6 BECKER FARM ROAD

(C) CITY: ROSELAND

(D) STATE: NEW JERSEY

(E) COUNTRY: USA

(F) ZIP: 07068

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5 INCH DISKETTE

(B) COMPUTER: IBM PS/2

(C) OPERATING SYSTEM: MS-DOS

(D) SOFTWARE: WORD PERFECT 5.1

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE: Submitted herewith

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA

(A) APPLICATION NUMBER:

(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: FERRARO, GREGORY D.

(B) REGISTRATION NUMBER: 36,134

(C) REFERENCE/DOCKET NUMBER: 325800-160

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 201-994-1700

(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 360 BASE PAIRS

(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGCAGGCC TGATGACCAT AGTAACCAGC CTTCTGTTCC TTGGTGTCTG TGCCCACCAC 60
ATCATCCCTA CGGGCTCTGT GGTCATACCC TCTCCCTGCT GCATGTTCTT TGTTTCCAAG 120
AGAATTCCTG AGAACCGAGT GGTCAGCTAC CAGCTGTCCA GCAGGAGCAC ATGCCTCAAG 180
GGAGGAGTGA TCTTCACCAC CAAGAAGGGC CAGCAGTTCT GTGGCGACCC CAAGCAGGAG 240
TGGGTCCAGA GGTACATGAA GAACCTGGAC GCCAAGCAGA AGAAGGCTTC CCCTAGGGCC 300
AGGGCAGTGG CTGTCAAGGG CCCTGTCCAG AGATATCCTG GCAACCAAAC CACCTGCTAA 360

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 119 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR
(ii) MOLECULE TYPE: PROTEIN
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Gly Leu Met Thr Ile Val Thr Ser Leu Leu Phe Leu Gly Val
-20 -15 -10
Cys Ala His His Ile Ile Pro Thr Gly Ser Val Val Ile Pro Ser Pro
-5 1 5 10
Cys Cys Met Phe Phe Val Ser Lys Arg Ile Pro Glu Asn Arg Val Val
15 20 25
Ser Tyr Gln Lys Ser Ser Arg Ser Thr Cys Leu Lys Gly Gly Val Ile

```
                30                  35                  40
Phe Thr Thr Lys Lys Gly Gln Gln Phe Cys Gln Asp Pro Lys
Gln Glu

        45                  50                  55
Trp Val Gln Arg Tyr Met Lys Asn Leu Asp Ala Lys Gln Lys
Lys Ala

     60                  65                  70
Ser Pro Arg Ala Arg Ala Val Ala Val Lys Gly Pro Val Gln
Arg Tyr

75                  80                  85
  90
Pro Gly Asn Gln Thr Thr Cys
                95
```

WHAT IS CLAIMED IS:

1. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding an MCP-4 polypeptide having the deduced amino acid sequence of Figure 1 or a fragment, analog or derivative of said polypeptide;

(b) a polynucleotide encoding an MCP-4 polypeptide having the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 75703 or a fragment, analog or derivative of said polypeptide.

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 1 wherein the polynucleotide is genomic DNA.

5. The polynucleotide of Claim 2 wherein said polynucleotide encodes MCP-4 having the deduced amino acid sequence of Figure 1.

6. The polynucleotide of Claim 2 wherein said polynucleotide encodes the MCP-4 polypeptide encoded by the cDNA of ATCC Deposit No. 75703.

7. The polynucleotide of Claim 1 having the coding sequence of MCP-4 as shown in Figure 1.

8. The polynucleotide of Claim 2 having the coding sequence of MCP-4 deposited as ATCC Deposit No. 75703.

9. A vector containing the DNA of Claim 2.

10. A host cell genetically engineered with the vector of Claim 9.

11. A process for producing a polypeptide comprising: expressing from the host cell of Claim 10 the polypeptide encoded by said DNA.

12. A process for producing cells capable of expressing a polypeptide comprising genetically engineering cells with the vector of Claim 9.

13. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having MCP-4 activity.

14. A polypeptide selected from the group consisting of (i) an MCP-4 polypeptide having the deduced amino acid sequence of Figure 1 and fragments, analogs and derivatives thereof and (ii) an MCP-4 polypeptide encoded by the cDNA of ATCC Deposit No. 75703 and fragments, analogs and derivatives of said polypeptide.

15. The polypeptide of Claim 14 wherein the polypeptide is MCP-4 having the deduced amino acid sequence of Figure 1.

16. An antibody against the polypeptide of claim 14.

17. An agonist to the polypeptide of claim 14.

18. An antagonist/inhibitor against the polypeptide of claim 14.

19. A method for the treatment of a patient having need of MCP-4 comprising: administering to the patient a therapeutically effective amount of the polypeptide of claim 14.

20. A method for the treatment of a patient having need to inhibit MCP-4 comprising: administering to the patient a therapeutically effective amount of the antagonist/inhibitor of Claim 18.

21. A pharmaceutical composition comprising the polypeptide of Claim 14 and a pharmaceutically acceptable carrier.

22. The method of Claim 19 wherein said therapeutically effective amount of the polypeptide is administered by providing to the patient DNA encoding said polypeptide and expressing said polypeptide *in vivo*.

FIG. 1

```
1    ATGGCAGGCCTGATGACCATAGTAACCAGCCTTCTGTTCCTTGGTGTCTGTGCCCACCAC   60
     M  A  G  L  M  T  I  V  T  S  L  L  F  L  G  V  C  A  H  H

61   ATCATCCCTACGGGCTCTGTGGTCATACCCTCTCCCTGCTGCATGTTCTTTGTTTCCAAG  120
     I  I  P  T  G  S  V  V  I  P  S  P  C  C  M  F  F  V  S  K

121  AGAATTCCTGAGAACCGAGTGGTCAGCTACCAGCTGTCCAGCAGGAGCACATGCCTCAAG  180
     R  I  P  E  N  R  V  V  S  Y  Q  L  S  S  R  S  T  C  L  K

181  GGAGGAGTGATCTTCACCACCAAGAAGGGCCAGCAGTTCTGTGGCGACCCCAAGCAGGAG  240
     G  G  V  I  F  T  T  K  K  G  Q  Q  F  C  G  D  P  K  Q  E

241  TGGGTCCAGAGGTACATGAAGAACCTGGACGCCAAGCAGAAGAAGGCTTCCCCTAGGGCC  300
     W  V  Q  R  Y  M  K  N  L  D  A  K  Q  K  K  A  S  P  R  A

301  AGGGCAGTGGCTGTCAAGGGCCCTGTCCAGAGATATCCTGGCAACCAAACCACCTGCTAA  360
     R  A  V  A  V  K  G  P  V  Q  R  Y  P  G  N  Q  T  T  C  *
```

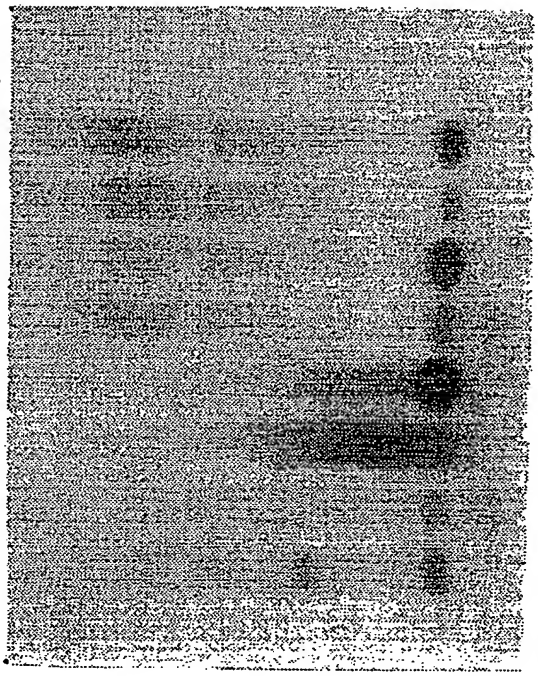
FIG. 2

```
  1 M.AGLMTIVTSLLFLGVCAHHIIPTGSVVIPSPCCMFFVSKRIPENRVVSYQ  51
    |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
  1 MQVPVMLLGLLFTVAGWSIHVLAQPDAVNAPLTCCYSFTSKMIPMSRLESYX  52

 52 LSSRSTCLKGGVIFTTKKGQQFCGDPKQEWVQRYMKNLDAKQKKASPRAR  101
    |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
 53 RITSSRCPKEAVVFVTKLKREVCADPKKEWVQTYIKNLDRNQMRSEPTTL  102

102 AVAVKGPVQRYPGNQTTC 119
    |  |  |  |  |  |  |
103 FKTASALRSSAPLNVHLTRKSEANASTTFSTTTSSTSVGVTSVTVN 148
```

FIG. 3

NORTHERN BLOT ANALYSIS OF MCP

1 2 3 4 5 6 7 8 9



1 = MONOCYTE 2 = ACTIVATED MONOCYTE 3 = T CELL

4 = ACTIVATED T CELL 5 = JURAT 6 = ACTIVATED JURKAT

7 = SUPT 8 = ACTIVATED SUPT

FIG. 5

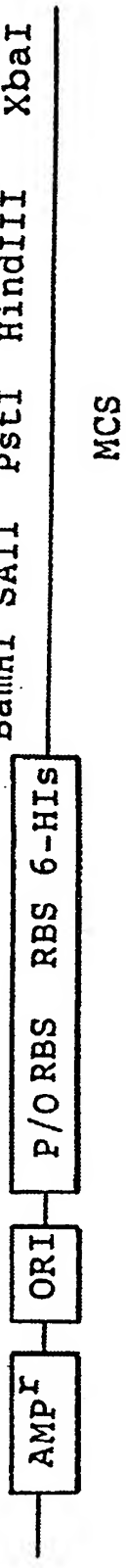
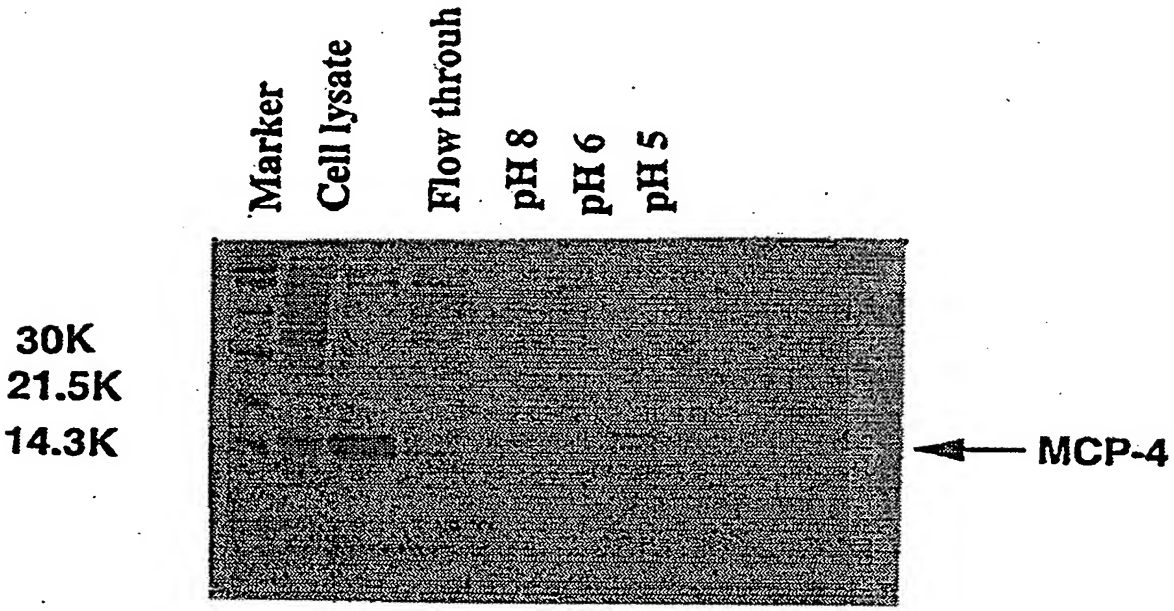


FIG. 4

Expression of MCP-4 in E. coli



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05384

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :536/23.5; 435/320.1, 252.3, 69.1; 530/380; 514/2; 424/93A
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5; 435/320.1, 252.3, 69.1; 530/380; 514/2; 424/93A

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG - Biotech files; APS; GENEMBL databases

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Biochemical and Biophysical Research Communications, Volume 167, Number 3, issued 30 March 1990, Decock et al, "Identification of the Monocyte Chemotactic Protein from Human Osteosarcoma Cells and Monocytes: Detection of a Novel N-Terminally Processed Form", pages 904-909, see entire document.	14, 15
Y	Biochemical and Biophysical Research Communications, Volume 169, Number 2, issued 15 June 1990, Shyy et al, "Structure of Human Monocyte Chemotactic Protein Gene and Its Regulation by TPA", pages 346-351, see entire document.	1-15

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 29 JULY 1994	Date of mailing of the international search report 23 AUG 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Jill Warden for ELIZABETH C. KEMMERER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05384

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proceedings f the National Academy of Sciences USA, Volume 86, issued March 1989, Robinson et al, "Complete Amino Acid Sequence of a Human Monocyte Chemoattractant, a Putative Mediator of Cellular Immune Reactions", pages 1850-1854, see entire document.	1-15
Y	FEBS Letters, Volume 244, Number 2, issued February 1989, Yoshimura et al, "Human Monocyte Chemoattractant Protein-1 (MCP-1): Full-Length cDNA Cloning, Expression in Mitogen-Stimulated Blood Mononuclear Leukocytes, and Sequence Similarity to Mouse Competence Gene JE", pages 487-493, see entire document.	1-15
Y	European Journal of Biochemistry, Volume 199, issued 1991, Van Damme et al, "Production and Identification of Natural Monocyte Chemotactic Protein from Virally Infected Murine Fibroblasts", pages 223-229, see entire document.	1-15
Y	Proceedings of the National Academy of Sciences USA, Volume 85, issued June 1988, Rollins et al, "Cloning and Expression of *JE*, a Gene Inducible by Platelet-Derived Growth Factor and Whose Product has Cytokine-Like Properties", pages 3738-3742, see entire document.	1-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05384

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

C07H 17/00; C12N 15/00, 1/20; C12P 21/06; C07K 15/00; A01N 37/18, 63/00

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-15 and 19-22, drawn to nucleic acids, vectors, host cells, a process of producing a polypeptide, polypeptides, a method of treating disease with polypeptides, and a pharmaceutical composition comprising polypeptides.

II. Claim 16, drawn to an antibody.

III. Claims 17 and 18, drawn to agonists, antagonists, and/or inhibitors.

Inventions I, II, and III represent separate inventive concepts. Invention I relates to the general inventive concept of recombinant protein production, and the use of the recombinantly produced protein to treat disease. Invention II relates to the general inventive concept of an antibody, which is useful as a diagnostic tool, or in studying the expression pattern of the polypeptide in its native host environment. Invention III relates to the general inventive concept of substances which regulate the expression of the polypeptide, such substances being useful to externally control expression.

Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single general inventive concept.